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ARTICLE

The Genetic Relationship between Anadromous and Resident *Oncorhynchus mykiss* at a Putative Barrier with Implications for Habitat Improvement

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Abstract

Big Bear Creek in the Potlatch River system, Idaho, contains an Endangered Species Act-listed wild population of steelhead *Oncorhynchus mykiss* (anadromous Rainbow Trout) that is the focus of extensive habitat restoration actions intended to increase population abundance. Both anadromous and resident *O. mykiss* populations occur in the Big Bear Creek drainage; however, anadromous individuals are known to spawn and rear only in the lower drainage, whereas putative resident fish occur in the headwaters. Big Bear Falls is a potential upstream migration barrier that is located between the two populations. *Oncorhynchus mykiss* of unknown origin are present within 1 km above the falls. We used analyses of genetic diversity, structure, and sibship reconstruction to determine whether Big Bear Falls limited the movement of steelhead to headwater areas. Known resident *O. mykiss* were sampled in headwater areas, known anadromous adults were sampled at a weir in the lower drainage, and juveniles of unknown life history were collected from areas below and above the falls. Allele frequency data indicated that anadromous and headwater resident populations were highly differentiated and that exchange of genetic material between populations was limited. However, juvenile *O. mykiss* in the above-waterfall collection were found to be the offspring or recent descendants of steelhead that successfully navigated above Big Bear Falls, demonstrating that the falls is not a complete barrier. We also identified evidence of limited downstream gene flow, suggesting that resident fish contributed genetic material to the downstream anadromous population. This study documented the successful passage of adult steelhead above Big Bear Falls and the downstream movement of resident fish from the headwaters. However, uncertainties still exist regarding the annual variability in passage and the habitat limitations that impact *O. mykiss* utilization of areas immediately above the falls.

A variety of anthropogenic alterations within the Snake River basin and downstream Columbia River over the past 100 years has contributed to the decline of steelhead *Oncorhynchus mykiss* (anadromous Rainbow Trout) runs (Raymond 1988; Tabor et al. 1993; Williams et al. 1999). As a result, Snake River steelhead were listed as threatened under the Endangered Species Act (ESA) in 1997 (Endangered Species Act of 1973; NMFS 1997). Since this ESA

listing, extensive research has been conducted involving management of the main-stem Snake River–Columbia River hydropower system (Williams et al. 1999; Matter and Sandford 2003; Waples et al. 2008). Main-stem actions have focused on improving the survival of downstream-migrating juvenile salmon and steelhead through the hydropower system. Recently, additional focus has been placed on stream restoration within freshwater spawning and

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rearing habitats as a means to improve overall population numbers (NOAA 2008).

Stream restoration is an evolving science with an increased emphasis on projects in areas that are occupied by ESA-listed species (Roni et al. 2002). Stream restoration often includes active physical manipulation of the existing stream habitat, such as the addition of large woody debris, the removal of levees to restore floodplain connectivity, or attempts to narrow an overly widened channel (Abbe and Montgomery 1996; Johnson et al. 2005). Passive restoration techniques include alteration of domestic grazing regimes and can be conducted on neighboring landscapes to reduce sediment or decrease water temperatures (Kauffman et al. 1997). Off-channel activities include road decommissioning or riparian fencing. The benefits to the target stream species from instream or offsite habitat restoration, such as decreased water temperatures or decreased sedimentation, may take years to be fully realized.

Barrier removal is a common and attractive habitat restoration strategy because it provides a benefit to the target species within a very short time. Barrier removal generally entails the removal of an anthropogenic structure to restore fish passage, thereby allowing fish to recolonize the habitat. Barrier removal can mitigate for fish loss of access to breeding and rearing habitat in the upstream reach and can restore hydrological and ecological function to the downstream reach (O'Hanley 2011). A less common strategy is installation of anthropogenic barriers to maintain a species' isolation from invasive nonnative competitors (Thompson and Rahel 1998; Novinger and Rahel 2003).

Oncorhynchus mykiss encompasses a diversity of life history types, including a freshwater resident form (Rainbow Trout) and an anadromous form (steelhead). All *O. mykiss* are born in freshwater, but in systems with access to the ocean, steelhead may smolt and out-migrate to take advantage of the more productive saltwater environment (Kendall et al. 2015 and references therein). Within drainages containing both life history forms of *O. mykiss*, it is important to understand how barrier removal or creation may affect interactions and reproductive exchange between the two forms. Through barrier removal, available habitat may be gained by either life history form. However, if the barrier was formed naturally, an unintended negative consequence of barrier removal is that the genetic isolation of divergent groups could be disrupted, especially for cases in which a resident population occurs above the barrier (Van Doornik et al. 2013).

Steelhead in Big Bear Creek, a tributary of the Potlatch River in Idaho, belong to the lower Clearwater River population of the Snake River Distinct Population Segment (ICTRT 2003). Steelhead monitoring has documented that density-dependent factors exist within the drainage and that steelhead production is limited by habitat availability (Bowersox et al. 2012). Some stream reaches within the lower Potlatch River drainage, including Big Bear Creek, become dewatered by late summer, concentrating the rearing juvenile steelhead into

watered reaches and subsequently increasing density-dependent factors in the rearing habitat (Bowersox et al. 2012). Therefore, an increase in the available habitat, even into similarly impacted reaches, will result in greater availability of habitat for rearing juvenile steelhead. Current habitat restoration efforts within the Potlatch River drainage are intended to increase available rearing habitat, and barrier removal has been identified as a high-priority restoration activity (Resource Planning Unlimited 2007).

Big Bear Falls is a two-chute waterfall that potentially acts as a barrier to upstream migration of adult steelhead in Big Bear Creek. An historic account from a resident in the upper Big Bear Creek drainage mentioned steelhead being caught above the falls in the past (R. Johnston, U.S. Forest Service, personal communication). However, anthropogenic changes in the uplands surrounding the Big Bear Creek drainage have significantly altered the springtime hydrograph and associated stream morphology during the period of adult steelhead migration, and these changes may affect current fish passage success. The falls encompasses a series of cascades approximately 33 m in length and 12 m in height (see Figure 1). The largest drops in the cascade are 2.7 and 3.6 m high. Wild steelhead are present throughout Big Bear Creek below the falls. In addition, 20 km of potential spawning and rearing habitat are available above the falls, and this area contains a lower density of juvenile *O. mykiss* than documented elsewhere in the Potlatch River drainage. In terms of flow regime, instream water temperature, and substrate, the habitat within this 20-km reach is similar to steelhead-occupied reaches below the falls. If passage has been reduced due to alteration of environmental conditions, then providing or improving passage for steelhead at the waterfall could effectively double the amount of habitat available within Big Bear Creek. However, the extent to which the falls influences *O. mykiss* passage and distribution in Big Bear Creek has not been explored, and the degree of genetic isolation and interactions between *O. mykiss* with anadromous and resident life histories is currently unknown.

In addition to the different *O. mykiss* life histories present within Big Bear Creek, nonnative, hatchery-origin coastal Rainbow Trout *O. mykiss irideus* may have become established in the Big Bear Creek headwaters due to historical stocking of private water bodies within the upper portions of the drainage. The resident *O. mykiss* population in the creek's headwaters may have become established in association with these activities. Within the Columbia River basin, the Redband Trout *O. mykiss gairdneri* is the native Rainbow Trout subspecies of the Snake River basin and Idaho, whereas the coastal Rainbow Trout is native to areas west of the Cascade Range in Oregon and Washington (Behnke 2002; Blankenship et al. 2011; Matala et al. 2014). Both the Redband Trout and the coastal Rainbow Trout lineages exhibit resident and anadromous life histories. Coastal Rainbow Trout introgression with native Redband Trout (e.g., Williams et al. 1996) after barrier

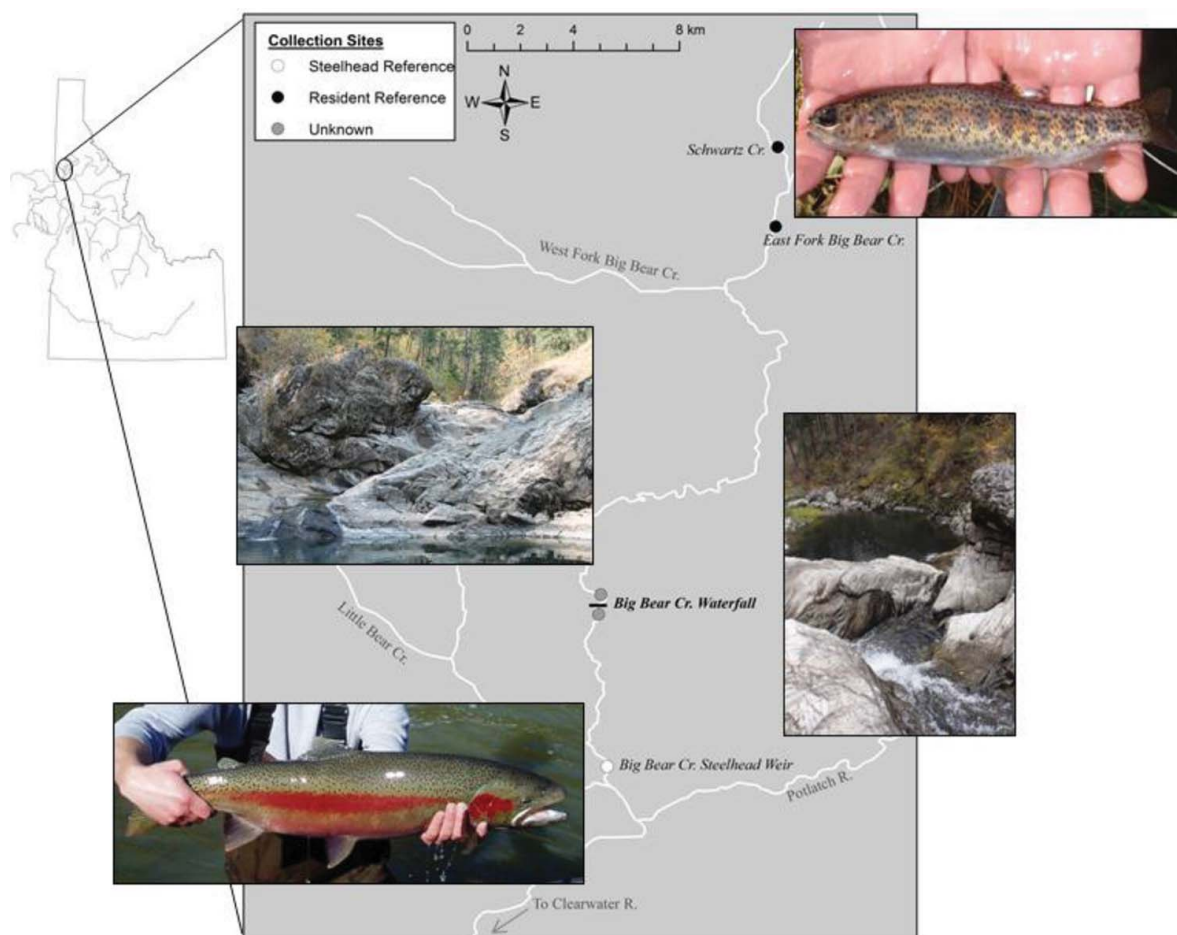


FIGURE 1. Map of the Big Bear Creek study area in Idaho. Lower left photo is representative of the returning adult anadromous *Oncorhynchus mykiss* that were captured at the weir on lower Big Bear Creek. Big Bear Falls is depicted in the middle left and middle right photos (juvenile samples of unknown life history type were taken approximately 200 m above and below the falls). Upper right photo depicts a resident *O. mykiss* individual that was captured during electrofishing surveys in the Big Bear Creek headwaters.

removal is a concern and may confound evaluations of genetic structure (Ackerman et al. 2012).

Partners implementing habitat restoration in the Big Bear Creek drainage have debated the best restoration approach given the uncertainties about fish passage at Big Bear Falls; questions include whether the falls is affecting *O. mykiss* distribution, or whether other environmental factors (e.g., above the falls) are limiting production. To inform habitat restoration actions within the Big Bear Creek drainage a better understanding of the relationship between resident and anadromous *O. mykiss* in the drainage is required. The overarching goal of this study was to determine whether Big Bear Falls has influenced the distribution of *O. mykiss* within the Big Bear Creek drainage. To this end, our study objectives were fourfold: (1) to evaluate the degree of reproductive isolation between resident *O. mykiss* present in headwater tributaries above the falls and known anadromous *O. mykiss* (i.e., steelhead) that occur downstream of the falls; (2) determine whether genetic differentiation, if present, is related to

out-of-basin transfers of nonnative coastal Rainbow Trout into Big Bear Creek; (3) establish whether Big Bear Falls is a complete barrier to the upstream migration of adult steelhead and determine the life history type (resident, anadromous, or mixed) of juvenile *O. mykiss* captured both below and above the falls; and (4) determine the potential contributions of headwater resident *O. mykiss* populations to steelhead production downstream of the falls.

METHODS

Field data collection.—Tissue samples were taken from a total of 268 fish within Big Bear Creek, which is located in Latah County, Idaho (Table 1). Collections represented three groups: (1) reference resident *O. mykiss* that were sampled in headwater tributaries, (2) reference anadromous *O. mykiss* that were sampled below Big Bear Falls, and (3) individuals of unknown life history that were collected proximate to (both above and below) the falls (Figure 1). All captured age-classes

TABLE 1. Collection year, sample size (n), life stage, life history type, summary of deviations from Hardy–Weinberg equilibrium (HWE) expectations (before correction for multiple tests; heterozygote excess, heterozygote deficiency, and percentage of single-nucleotide polymorphisms [SNPs] that were affected), expected heterozygosity (H_E), allelic richness (AR), and percentage of polymorphic SNPs for five collections of *Oncorhynchus mykiss* from the Big Bear Creek drainage, Idaho.

Collection	Collection year	n	Life stage	Life history type	HWE deviations ^a				H_E (%)	AR	Percent polymorphic SNPs
					Heterozygote excess	Heterozygote deficiency	SNPs (%)				
East Fork Big Bear Creek	2011	48	Mixed	Resident	1	9	5.3		28.1	1.856	88.2
Schwartz Creek	2011	52	Mixed	Resident	0	6	3.2		27.2	1.834	87.7
Above waterfall	2011, 2012	32	Juvenile	Unknown	13	7	10.7		20.2	1.690	74.3
Below waterfall	2011	36	Juvenile	Unknown	2	7	4.8		30.2	1.929	97.3
Big Bear Creek (weir)	2007, 2008, 2010, 2011	99	Adults	Anadromous	2	5	3.7		31.2	1.951	99.5

^aThere was a single significant deviation from HWE expectations after correction for multiple tests; the deviation was due to heterozygote excess in the SNP OMS00058 within the above-waterfall collection.

and size-classes were included in the resident and unknown life history collections, which were obtained by using single-pass electrofishing. Headwater resident *O. mykiss* were collected from East Fork Big Bear Creek ($n = 48$ fish) and Schwartz Creek ($n = 52$) on October 27, 2011. The area below Big Bear Falls (hereafter, “below waterfall”) was sampled on October 28, 2011 ($n = 36$ fish); the area above the falls (hereafter, “above waterfall”) was sampled on October 28, 2011 ($n = 3$), and on September 24, 2012 ($n = 30$). The above-waterfall and below-waterfall collections were obtained from reaches over 200 m that included multiple habitat breaks. Fish were anesthetized by using tricaine methanesulfonate (MS-222), individual FLs (nearest 1 mm) were recorded, a tissue sample (caudal fin clip) was taken from each individual, and the fish were then released back into the stream. Adult steelhead were collected at a weir in lower Big Bear Creek, which was operated as part of an intensive monitoring program within the creek from 2005 to 2012. Samples from the weir had previously been genotyped to document the genetic structure of steelhead throughout the Snake River basin for purposes of genetic stock identification (Ackerman et al. 2014; Matala et al. 2014). For the present study, we included genotype data from 99 adult steelhead that were sampled during 2007, 2008, 2010, and 2011.

Laboratory protocol and data processing.—All samples were genotyped at 191 single-nucleotide polymorphisms (SNPs) and were subject to a Y-chromosome-specific assay that differentiates sex in *O. mykiss* (Campbell et al. 2012). The DNA was extracted using Nexttec Genomic DNA Isolation Kits from XpressBio (Thurmont, Maryland). Prior to DNA amplification of SNP loci using primer–probe sets (fluorescent tags), an initial PCR preamplification was implemented with whole genomic DNA to jumpstart SNP amplification by increasing the copy number of target DNA regions. The PCR conditions for the preamplification step were as follows: an initial denaturation at 95°C for 15 min, followed by 14 cycles of 95°C for 15 s and 60°C for 4 min, and ending with a final dissociation step at 4°C. Genotyping was performed using Fluidigm 96.96 Dynamic Array Integrated Fluidic Circuits (IFCs). For each genotyping run, 96 samples (including an extraction negative control, a PCR negative control, and a PCR positive control) and 96 TaqMan SNP assays were hand-pipetted onto the 96.96 IFCs. Sample cocktail and SNP assay cocktail recipes are available from the corresponding author upon request. Each 96.96 IFC was pressurized, and a Fluidigm IFC Controller HX was used to load the sample mixture and SNP assays into the IFC. Amplification of SNPs on the 96.96 IFCs was performed using the Fluidigm FC1 Cycler. The final SNP amplification protocol was as follows: a thermal mixing step at 70°C for 30 min and 25°C for 10 min; a hot-start step at 95°C for 60 s, followed by 50 cycles of 95°C for 5 s and 58°C for 25 s; and a final cool-down step at 25°C for 10 s. The IFCs were imaged on a Fluidigm EP1 and were analyzed and scored using Fluidigm SNP

Genotyping Analysis software. Genomic DNA extraction and amplification methods and SNP genotyping using multiplex 5'-nuclease reactions are described in detail by Seeb et al. (2009).

The 191-SNP panel included three SNPs (*Ocl_gshpx-357*, *Omy_Omyclmk438-96*, and *Omy_myclar404-111*) that were designed to identify potential *O. mykiss* × Cutthroat Trout *O. clarkii* hybrids. Any fish that was flagged as a Cutthroat Trout or hybrid for at least two of the three SNPs was removed from the data set; the three SNPs were then removed from all subsequent analyses. Ackerman et al. (2014) identified two SNPs (*Omy_GHSR-121* and *Omy_mapK3-103*) that exhibited linkage disequilibrium in a majority of collections from Snake River steelhead populations. In that study, *Omy_mapK3-103* was the less informative of the locus pair and was dropped from analyses; *Omy_mapK3-103* was also removed from the present study. In total, 187 SNPs were used in all subsequent analyses unless otherwise noted.

We used a function written in R software (R Development Core Team 2009) to identify duplicate-sampled individuals; we identified any pairs of individuals that had duplicate genotypes for at least 182 of the 187 SNPs (we allowed up to five mismatches).

Standardized genotypes were stored on a Progeny (www.progenygenetics.com) database server housed at the Eagle Fish Genetics Laboratory in Idaho. The final 187-SNP data set used for this study was also uploaded to a standardized genetic data repository (www.FishGen.net).

Testing for Hardy–Weinberg equilibrium.—Tests for deviation from Hardy–Weinberg equilibrium (HWE) expectations were performed across all loci for each population by using exact *P*-values calculated via the complete enumeration method (Louis and Dempster 1987) implemented in GENEPOP version 4.1.2 (Rousset 2008). Critical values ($\alpha = 0.05$) were adjusted for multiple tests by using a step-down sequential Bonferroni correction. For each collection, we report the number of SNPs deviating from HWE that exhibited either an excess of heterozygotes or a deficit of heterozygotes (F_{IS} ; Weir and Cockerham 1984) both before and after Bonferroni correction for multiple tests. We examined for deviations from HWE that might suggest (1) a Wahlund effect (heterozygote deficiency; Wahlund 1928) indicating subpopulation structure within the sample; or (2) few breeders contributing to the next generation, resulting in an excess of sibling associations within the sample (heterozygote excess; Rasmussen 1979; Pudovkin et al. 1996).

Genetic diversity and structure.—We used GenAlEx version 6.5 (Peakall and Smouse 2006) to evaluate the genetic diversity within each collection and the genetic structure among collections to elucidate the degree of reproductive isolation between resident *O. mykiss* collected in upper Big Bear Creek and anadromous individuals collected from the lower portion of the drainage. Using GenAlEx, we calculated the mean expected heterozygosity (H_E) across SNPs and the

percentage of polymorphic SNPs within each collection as measures of genetic diversity. In addition, allelic richness (AR) was calculated for each population by using FSTAT version 2.9.3.2 (Goudet 1995, 2001). Each population's AR was calculated as the average across loci. We tested for differences in mean H_E and AR among the five *O. mykiss* collections by using a Kruskal–Wallis test (Kruskal and Wallis 1952); Dunn's test (Dunn 1964) was then used to conduct comparisons between pairs of collections. Pairwise genetic differentiation index F_{ST} values were calculated to measure genetic structure among collections, and a multivariate principal coordinates analysis (PCoA) based on the matrix of pairwise F_{ST} values was performed in GenAlEx to visualize variation in the data. We used permutation tests (999 replicates) in GenAlEx to assess the statistical significance of genetic differentiation between pairs of collections.

Evaluating potential introgression with coastal Rainbow Trout.—To identify whether our collections showed evidence of introgression with nonnative coastal Rainbow Trout, we used STRUCTURE version 2.3.4 (Pritchard et al. 2000) to estimate the inferred ancestry of each individual and each collection to two populations (i.e., $K = 2$ clusters) representing (1) native Redband Trout and (2) nonnative coastal Rainbow Trout. Because coastal Rainbow Trout are highly differentiated from native Redband Trout, intraspecific introgression should be identified with relative ease (e.g., Matala et al. 2014). The anadromous collection obtained at the Big Bear Creek weir served as a reference *O. mykiss gairdneri* collection. For coastal Rainbow Trout reference collections, we used four hatchery strains (Mt. Lassen–Hildebrand: $n = 47$ fish; Mt. Lassen–Donaldson: $n = 46$; Hayspur–R9: $n = 22$; Hayspur–Kamloops: $n = 23$) that were previously genotyped with the same SNP panel used here; the strains were representative of those previously stocked in the Potlatch River drainage. A 100,000-iteration burn-in and 250,000 iterations of the Markov chain–Monte Carlo were used to estimate group membership to $K = 2$ clusters for each collection. Default parameters of admixture and correlated allele frequencies were used.

Sibship reconstruction.—Sibship reconstruction methods assign sibling relationships among a single sample of individuals. The maximum likelihood method developed by Wang and Santure (2009) as implemented in COLONY version 2.0 (Wang 2004; Jones and Wang 2010) was used to assign sibship among juvenile *O. mykiss* in the above-waterfall and below-waterfall collections, which were run in a single analysis. Performing simultaneous sibship reconstruction for juveniles sampled below and above Big Bear Falls allowed us to evaluate passage at the falls; identification of a full-sibling dyad with one sibling above the falls and one sibling below would provide evidence for successful downstream passage. We also performed two additional COLONY analyses in which the above-waterfall and below-waterfall collections were considered separately. Separate evaluations of sibling relationships facilitated estimation of the effective population

size (N_E) of the parent generation for each collection by using a single sample of offspring (Wang 2009; Waples and Waples 2011). In all COLONY analyses, we allowed for a polygamous mating system (likely the most appropriate system for natural populations of *O. mykiss*), but we ignored any estimated half-sibling relationships. Previous evaluations based on 192 SNPs from known populations of *O. mykiss* have demonstrated that half-sibling relationships estimated in COLONY are inaccurate. However, despite inaccurate half-sibling reconstruction, estimates of N_E can be accurate if the sample size is sufficient (M. W. Ackerman, unpublished data). We acknowledge that our sample sizes for the above-waterfall and below-waterfall collections may have been too low to provide accurate N_E estimates for those populations. However, we were primarily interested in the relative difference in N_E estimates between the two collections, as sample sizes were similar (above waterfall: $n = 32$ fish; below waterfall: $n = 36$ fish) and sampling methods were the same. We used the statistical framework for a mating system with no inbreeding and an error rate of 0.0005 alleles/locus to account for miscellaneous genotyping errors (e.g., mutations, null alleles, and genotyping/scoring errors). Scale samples and ages were not available for fish that were collected from areas adjacent to the falls.

Inferences of individual group membership.—We hypothesized that STRUCTURE would identify $K = 2$ clusters (representing resident and anadromous groups) as the most appropriate number of populations in Big Bear Creek. To test this hypothesis, we first used STRUCTURE to evaluate K -values from 1 to 5 (20 iterations per K -value). The most likely number of clusters was determined by using the ΔK approach (Evanno et al. 2005) as implemented in STRUCTURE HARVESTER (Earl and von Holdt 2012). We chose to evaluate K -values greater than 2 (and up to 5) due to concerns that juveniles in the above-waterfall collection might represent a previously uncharacterized population occurring upstream of Big Bear Falls but below our reference resident collections. Default parameters of admixture and correlated allele frequencies were used; these parameters account for recent gene flow among populations and allow for some flexibility in linkage disequilibrium within populations. These default settings are most flexible for dealing with real biological phenomena (Pritchard et al. 2010) and therefore are likely the most appropriate settings for application to *O. mykiss*. A 100,000-iteration burn-in length and 250,000 iterations of the Markov chain–Monte Carlo were used to estimate group membership for each fish.

As expected, the initial Bayesian cluster analysis with STRUCTURE strongly suggested that the most likely K -value was 2 ($\Delta K = 1,146$), corresponding to (1) resident populations in the Big Bear Creek headwaters and (2) the anadromous population below Big Bear Falls. After determining the appropriate K , we then estimated each individual's inferred membership coefficient (q) to the two clusters. We hypothesized that (1) reference resident *O. mykiss* collected in the Big Bear Creek headwaters would have strong assignment to

the resident group (q_r); and (2) adult *O. mykiss* collected at the weir on Big Bear Creek would have strong assignment to the anadromous group (q_a). We were primarily interested in determining whether the juveniles belonging to the above-waterfall and below-waterfall collections would then be assigned to the resident or anadromous group. For each individual fish i , STRUCTURE estimated the proportion q of the genome that originated from each cluster K . For the second STRUCTURE run, we performed five iterations at $K = 2$ while using the same settings as above; we then calculated the average q for each individual across iterations. For each collection, mean membership (Q) was calculated as q averaged across individuals. We chose to exclude SNPs with a minor allele frequency (MAF) difference less than 10%, as many of the SNPs used here were developed to differentiate between coastal Rainbow Trout and Redband Trout lineages and for research across the Columbia River basin and Pacific Rim range of *O. mykiss* and therefore were not particularly useful in evaluating structure within Big Bear Creek. We first used GenAIEx to calculate the allele frequencies for (1) the pooled reference resident collections (Schwartz and East Fork Big Bear creeks) and (2) the reference anadromous collection from the Big Bear Creek weir. We then calculated the MAF difference for each SNP between the two reference groups, and only those SNPs with an MAF difference of at least 10% were included in the SNP panel for this STRUCTURE analysis.

RESULTS

Deviations from Hardy–Weinberg Equilibrium Expectations

Out of 800 tests, there was one significant deviation from HWE expectations after we applied the Bonferroni correction for multiple tests (across loci and populations, excluding SNPs fixed within collections). The single HWE deviation was due to an excess of heterozygotes at *OMS00058* within the above-waterfall collection. Before correction for multiple tests, 52 of the 800 tests indicated significant deviations; of those, 20 deviations (10.7% of the 187 SNPs) also occurred in the above-waterfall collection (13 deviations due to

heterozygote excess; 7 deviations due to heterozygote deficiency). The heterozygote excess at 13 SNPs (including *OMS00058*, which was still significant after Bonferroni correction) could indicate that few breeders contributed to the above-waterfall sample or that the above-waterfall collection contained a large number of siblings. This is further addressed in the sibship reconstruction section below.

Genetic Diversity and Structure

Kruskal–Wallis tests to examine for differences in H_E ($P < 0.0001$) and AR ($P < 0.0001$) indicated significant differences in genetic diversity among the five *O. mykiss* collections. Dunn's tests were then conducted to evaluate significant differences in H_E and AR between pairs of collections; those results are summarized in Table 2. The reference resident collections exhibited lower genetic diversity than the reference anadromous (weir) collection (Table 1). Measures of average H_E (across SNPs) were significantly lower for the East Fork Big Bear Creek ($H_E = 28.1\%$; Dunn's test: $P = 0.0428$) and Schwartz Creek ($H_E = 27.2\%$; $P = 0.0209$) collections than for the reference anadromous collection ($H_E = 31.2\%$). The average H_E of the reference anadromous collection was similar to that of the below-waterfall juvenile collection ($H_E = 30.2\%$; Dunn's test: $P = 0.2567$), whereas the above-waterfall collection had a markedly lower value ($H_E = 20.2\%$) than all other collections ($P \leq 0.0001$ for all comparisons). The reference resident collections also exhibited lower AR (East Fork Big Bear Creek: AR = 1.856; Schwartz Creek: AR = 1.834) than the reference anadromous collection (AR = 1.951), although the difference for the East Fork Big Bear Creek collection was not significant. The above-waterfall collection of juvenile *O. mykiss* also had significantly lower AR than all other collections ($P < 0.001$ for all comparisons). The AR for the below-waterfall collection (AR = 1.929) was similar to that of the reference anadromous collection ($P = 0.2508$). Similar patterns were observed for the percentage of polymorphic SNPs within each collection. Overall, polymorphism was observed in 88.2% of SNPs screened from the East Fork Big Bear Creek reference collection and in 87.7% of SNPs from the Schwartz Creek reference collection. In other words, over 10% of the

TABLE 2. Results of Dunn's tests examining for statistically significant differences in expected heterozygosity (below diagonal) and allelic richness (above diagonal) between pairs of *Oncorhynchus mykiss* collections from the Big Bear Creek drainage (NS = $P > 0.05$ [not significant]; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$). The primary life history type of each collection is identified in parentheses (A = anadromous; R = resident; U = unknown).

Collection	East Fork Big Bear Creek (R)	Schwartz Creek (R)	Above waterfall (U)	Below waterfall (U)	Big Bear Creek (A)
East Fork Big Bear Creek (R)	—	NS	****	*	NS
Schwartz Creek (R)	NS	—	***	**	*
Above waterfall (U)	****	****	—	****	****
Below waterfall (U)	NS	NS	****	—	NS
Big Bear Creek (A)	*	*	****	NS	—

TABLE 3. Matrix of pairwise genetic differentiation index F_{ST} values among *Oncorhynchus mykiss* collections from the Big Bear Creek drainage. The principal coordinates analysis depicted in Figure 2 is based on these values. All comparisons indicated significant differences (all $P < 0.001$). The primary life history type of each collection is identified in parentheses (A = anadromous; R = resident; U = unknown).

Collection	East Fork Big Bear Creek (R)	Schwartz Creek (R)	Above waterfall (U)	Below waterfall (U)
Schwartz Creek (R)	0.012	—	—	—
Above waterfall (U)	0.086	0.085	—	—
Below waterfall (U)	0.043	0.039	0.071	—
Big Bear Creek (A)	0.035	0.031	0.064	0.009

SNPs screened from the reference resident collections were fixed for one allele. Conversely, less than 3% of SNPs were fixed for one allele in the reference anadromous collection (99.5% of SNPs were polymorphic) and in the below-waterfall collection (97.3% of SNPs were polymorphic). In the above-waterfall collection of juveniles, greater than 25% of the SNPs were fixed for one allele.

All pairwise F_{ST} comparisons between collections were significant ($P \leq 0.001$ for all comparisons). However, the two reference resident collections (East Fork Big Bear and Schwartz creeks) showed little differentiation from each other ($F_{ST} = 0.012$; Table 3). Similarly, the below-waterfall collection showed little differentiation from the reference anadromous collection ($F_{ST} = 0.009$; Table 3). However, F_{ST} values between the two reference resident collections versus the

reference anadromous collection or the below-waterfall collection ranged from 0.031 to 0.043, suggesting little reproductive exchange between (1) *O. mykiss* that were collected below the falls and (2) Big Bear Creek headwater residents. The first and second axes of the PCoA explained 89.8% of the variation in the pairwise F_{ST} matrix (Figure 2). The PCoA demonstrated genetic similarity between the two reference resident collections as well as similarity between the reference anadromous collection and the below-waterfall collection. This result suggests that the juveniles collected below Big Bear Falls were either steelhead or recent descendants of steelhead. However, the above-waterfall collection of juveniles was an outlier on the PCoA plot and showed no similarity to either the reference resident collections or the anadromous (reference and below-waterfall) collections (Figure 2). Using sibship reconstruction

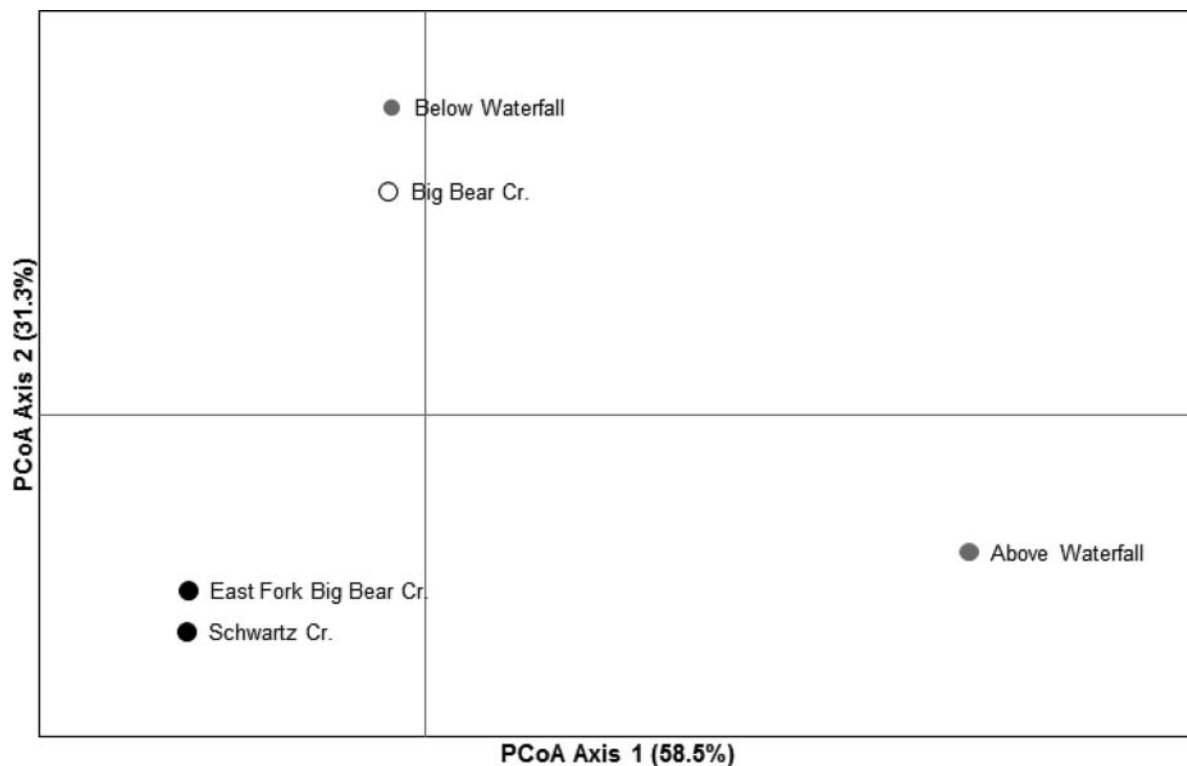


FIGURE 2. Principal coordinates analysis (PCoA) based on a pairwise matrix of genetic differentiation index F_{ST} values among study collections of *Oncorhynchus mykiss* from the Big Bear Creek drainage (see Table 3). Symbol shading represents life history type (black circle = reference resident; open circle = reference anadromous; gray circle = unknown life history).

(described below), we demonstrated that individuals in the above-waterfall collection were highly related and that the collection was unlikely to be representative of any population sampled within the Big Bear Creek drainage. Therefore, based solely on F_{ST} , it would be difficult to infer whether individuals in the above-waterfall collection were related to the resident populations or the anadromous population in the drainage.

Evaluating Potential Introgression with Coastal Rainbow Trout

Each of the five *O. mykiss* collections showed minimal or no evidence of introgression with nonnative coastal Rainbow Trout, suggesting that the resident populations in the Big Bear Creek headwaters were native Redband Trout. Each collection had group membership (i.e., results averaged across individuals) of 97.4% or greater to the Redband Trout lineage; three collections (East Fork Big Bear Creek, Schwartz Creek, and above waterfall) exceeded 99% group membership to that lineage. Three of the four reference collections of coastal Rainbow Trout had group membership of 98.9% or greater to the coastal Rainbow Trout lineage; the Hayspur–Kamloops strain had 94.5% group membership to that lineage.

Sibship Reconstruction

Among the 68 juveniles of unknown life history that were collected directly upstream and downstream of Big Bear Falls, we identified 30 unique family groups (Figure 3). None of the

family groups contained both an individual from the above-waterfall collection and an individual from the below-waterfall collection; thus, our sibling reconstruction methods failed to provide evidence for passage at Big Bear Falls. Of the 30 family groups identified, 23 groups were collected below Big Bear Falls and 7 groups were collected above the falls. Below the falls, the largest family group contained six full siblings, whereas 16 family groups contained only one individual. Above the falls, one family group (group 2 in Figure 3) contained 22 full siblings (i.e., estimated as having the same set of two parents). Based on the single sample of 32 individuals collected above the falls, the estimated N_E was 6 (95% confidence interval = 3–20). By comparison, the N_E for the below-waterfall collection was estimated at 23 (95% confidence interval = 14–42).

Inferences of Individual Group Membership

Results suggested that juveniles from the below-waterfall and above-waterfall collections were likely the offspring of steelhead or recent descendants of steelhead. We were confident in our individual assignments given the substantial genetic structure existing between the headwater collections of reference resident *O. mykiss* and the collection of anadromous fish from lower Big Bear Creek (Figure 4). Mean Q_r for the reference resident collections was 0.92 for the Schwartz Creek collection and 0.89 for the East Fork Big Bear Creek collection. The minimum q_r for a resident individual was 0.67; among the 100 individuals from the reference resident

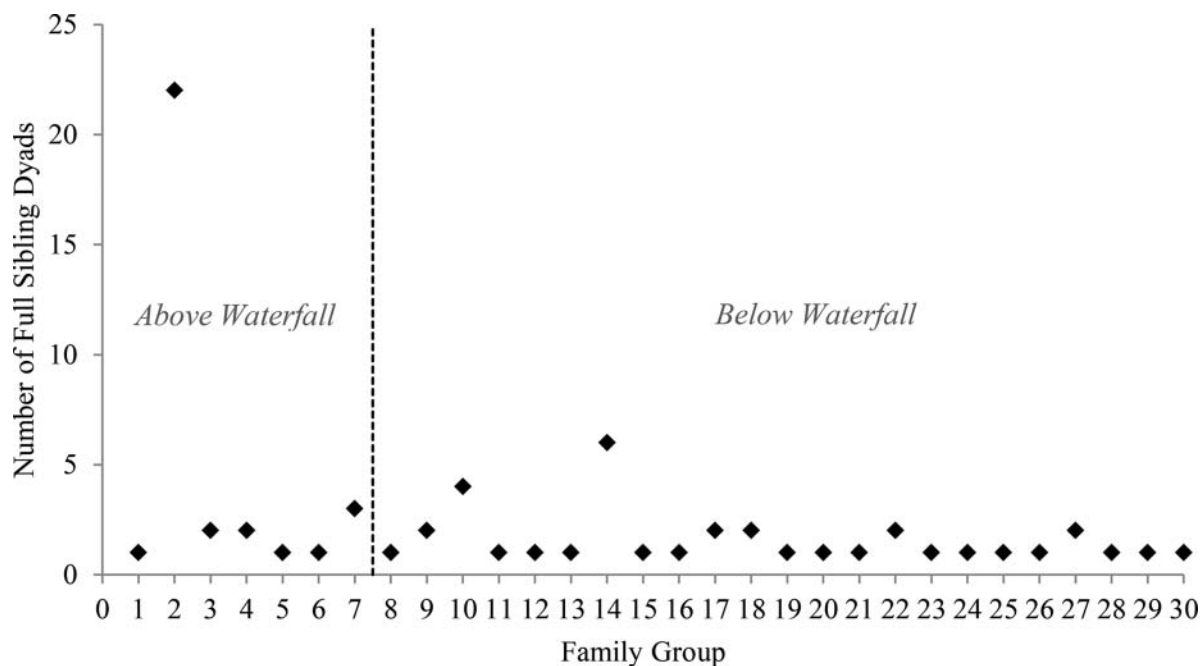


FIGURE 3. Number of full-sibling dyads in each *Oncorhynchus mykiss* family group identified by COLONY version 2.0 (Wang 2004; Jones and Wang 2010). Seven family groups were identified among juveniles collected above Big Bear Falls ($n = 32$); 30 family groups were identified among juveniles collected below the falls ($n = 36$). Family group 2 consisted of 22 full siblings.

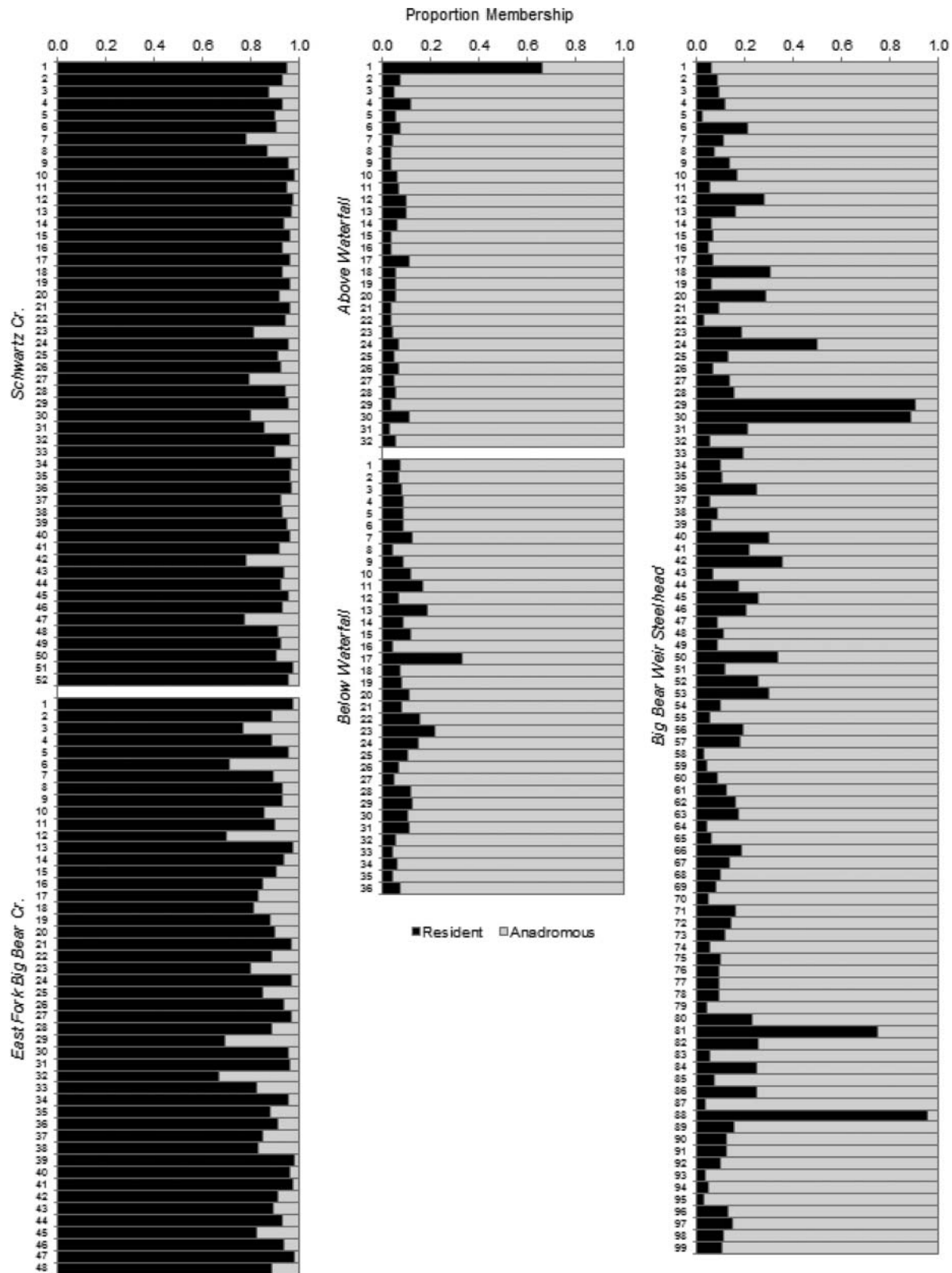


FIGURE 4. Proportional membership coefficients (q_i) for each sampled individual to inferred groups ($K = 2$ clusters) representing resident (black) and anadromous (gray) life histories of *Oncorhynchus mykiss* in the Big Bear Creek drainage. Analysis based on 187 single-nucleotide polymorphisms was performed in STRUCTURE version 2.3.4 (Pritchard et al. 2000), with default settings of admixture and correlated allele frequencies, a burn-in length of 100,000 iterations, and 250,000 iterations of the Markov chain–Monte Carlo. We performed five replicates of the analysis, and the q_i for each individual was averaged across replicates. The left panels depict data for the reference resident collections, the middle panels present data for juveniles of unknown life history (collected above and below Big Bear Falls), and the right panel illustrates data for the reference anadromous collection.

collections, 64 fish had a q_r value of at least 0.90. Within the reference anadromous collection, 83 of 99 individuals had a q_a value of 0.75 or greater (Figure 4). Interestingly, 5 of the 99 individuals from the reference anadromous collection had a q_r value of at least 0.50 and were thus assigned to resident ancestry; q_r values (in ascending order) for those five individuals were 0.50, 0.75, 0.89, 0.90, and 0.95.

All but one of the juvenile *O. mykiss* that were collected above or below Big Bear Falls had a majority q_a to the inferred anadromous ancestry (Figure 4). Of the 68 sampled juveniles, 67 fish had a q_a of at least 0.67 (mean = 0.92); 66 of those individuals had q_a values of 0.78 or greater. The single juvenile with majority membership to resident ancestry ($q_r = 0.66$) was collected above the waterfall (Figure 4, upper middle panel).

DISCUSSION

The results of our study better elucidate the relationship between the resident *O. mykiss* populations in the Big Bear Creek headwaters and the anadromous *O. mykiss* that spawn and rear in lower portions of the drainage. We demonstrated that significant reproductive isolation exists between the headwater resident populations and the steelhead occurring below Big Bear Falls. However, juveniles that were collected directly above the falls were found to be the offspring or recent descendants of steelhead, providing evidence that steelhead have successfully navigated the falls in recent generations. Furthermore, our results suggest that headwater resident populations are a source of limited gene flow to steelhead below the falls: five adult *O. mykiss* that were captured at the weir in lower Big Bear Creek were assigned to resident ancestry.

Despite the evidence that adult steelhead have successfully navigated the waterfall (albeit with limited success) and that headwater resident populations have contributed to the anadromous population, genetic differentiation persists between the anadromous and resident groups in Big Bear Creek. The reproductive isolation between Big Bear Creek's resident and anadromous populations was similar to the differentiation observed between steelhead from the Potlatch River drainage and those from the Middle Fork and South Fork of the Salmon River, Idaho—populations that spawn greater than 350 river kilometers (rkm) apart (Ackerman et al. 2014). Even though spawning and rearing habitat immediately above the falls displays environmental characteristics similar to those of the habitat below the falls, additional upstream habitat (a 6-km reach) between the falls and the headwater resident collection sites is severely degraded due to historical land use practices. It is possible that this area of degraded habitat plays a major role in the observed genetic isolation between resident and anadromous collections. In addition, spatial separation of the anadromous and resident groups may contribute to reproductive isolation, as Big Bear Falls was located approximately 26 km downstream of the resident *O. mykiss* collection site.

Multiple factors may lead to genetic isolation of upstream and downstream *O. mykiss* populations. Beyond the existence of Big Bear Falls, differences in springtime thermal regimes may promote reproductive isolation. Thermal regimes in upper Big Bear Creek are driven by snowmelt and therefore are significantly colder than the rain- and snowmelt-influenced regimes of the downstream canyon reaches. Peak spawn timing of adult steelhead in two Potlatch River tributaries, lower Big Bear Creek and the East Fork Potlatch River, is often separated by 3–4 weeks (Bowersox et al. 2011). Spawning occurs later in the East Fork Potlatch River due to its higher elevation and colder temperature regime. The elevation and water temperature profile at resident collection sites in upper Big Bear Creek is similar to that of the East Fork Potlatch River. Salmonids have adapted spawn timing throughout their range to maximize the survival of their progeny (Heggberget 1988; Bjornn and Reiser 1991). Webb and McLay (1996) documented significant variation in the spawn timing of Atlantic Salmon *Salmo salar* within Aberdeenshire Dee, Scotland. Spawning varied to the extent that spawn timing did not overlap for Atlantic Salmon in the upper and lower study reaches, which were situated approximately 100 rkm apart. Rapid changes in spawn timing have been linked to genetic divergence: Quinn et al. (2000) speculated that the separation of spawn timing served as a significant mechanism for genetic divergence between newly established populations of Chinook Salmon *O. tshawytscha* in New Zealand. Water temperatures in the upper Big Bear Creek drainage are consistently 1–2°C colder than those in the lower drainage during the peak period of steelhead spawning (March–April). Even with unimpeded passage at Big Bear Falls, resident and anadromous *O. mykiss* populations within the drainage might still maintain a level of genetic isolation due to temperature-mediated differences in spawn timing.

Genetic analysis in the present study demonstrated that successful passage of adult steelhead over Big Bear Falls does occur; however, passage limitations for adult steelhead at Big Bear Falls have been documented in recent years by using radiotelemetry techniques (Brian Knoth, Idaho Department of Fish and Game, personal communication). Therefore, we conclude that Big Bear Falls still plays some role in isolating the resident *O. mykiss* in upper Big Bear Creek from the anadromous individuals downstream, similar to conclusions reported by other researchers. Narum et al. (2008) sampled 21 locations throughout the Klickitat River, Washington, including locations below and above natural barriers and known *O. mykiss* life history types. Similar to our study, Narum et al. (2008) demonstrated that anadromous populations were generally characterized by high heterozygosity and genetic diversity, whereas resident populations had lower genetic diversity. In some cases, the difference in genetic diversity was attributable to reproductive isolation and reduced gene flow due to the presence of natural barriers. Reference resident collections from East Fork Big Bear and Schwartz creeks exhibited lower

values of H_E than the reference anadromous collection. Van Doornik et al. (2013) studied three rivers flowing into Hood Canal, Washington, two of which contained barriers to upstream migration; those authors demonstrated that *O. mykiss* population structure in the study rivers was influenced largely by the presence of barriers to upstream migration rather than by life history type.

Regarding the key question of whether Big Bear Falls poses an upstream migration barrier for adult steelhead, we found that it does not completely prohibit the upstream passage of anadromous adults. However, we documented much lower densities of juvenile *O. mykiss* in the habitat directly above the falls (<0.1 fish/100 m²) than directly below the falls (~ 10 fish/100 m²). Since our results showed that most juveniles in the above-waterfall collection were in fact either the offspring of steelhead or of recent steelhead ancestry, we speculated that most of them would out-migrate to the ocean as smolts. Therefore, if upstream passage at the falls is intermittent, juvenile densities may fluctuate, and the year-classes that are represented in the downstream reach (which is subject to annual steelhead spawning) may not all be represented in the reach above the falls, thereby resulting in a lower juvenile density within that reach. Although Big Bear Falls does not act as a complete barrier to upstream passage, we believe that the falls still plays a role in limiting upstream gene flow of *O. mykiss* in the drainage. Nevertheless, environmental factors occurring above the falls (and outside the scope of this study) may also influence the presence of *O. mykiss* above the falls.

Whether an individual *O. mykiss* undergoes smoltification or residualization is a trait under selection and is influenced both by genetics and by the local environment (Kendall et al. 2015). Anadromy contributes to the greater body size, energy stores, and fecundity of steelhead relative to the resident form, and these characteristics lead to increased reproductive success of steelhead when they return to the freshwater environment (typically their natal stream) to spawn (Hendry et al. 2004). However, anadromy comes at a cost. Steelhead experience greater physiological stress and a multitude of mortality risks (predation, increased fishing pressure, and hydropower system passage) that are associated with migration from freshwater to saltwater and back. In cases where anadromous and resident life history types occur in sympatry, studies have documented that resident parents can produce offspring that (1) migrate to the ocean to take advantage of the more productive saltwater environment and (2) successfully return to spawn as steelhead (Zimmerman et al. 2009; Courter et al. 2013; Van Doornik et al. 2013). Environmental conditions immediately above (within 20 km upstream of) the falls are similar to conditions in the area below the falls, which contains primarily anadromous *O. mykiss* and very few resident fish. Therefore, we would expect that the distribution of life history strategies among the *O. mykiss* spawning and rearing immediately above the falls would be similar to the distribution observed below the falls.

Despite the apparent limited reproductive exchange between the headwater resident populations and anadromous *O. mykiss* in lower Big Bear Creek, we found evidence that the upstream resident population contributes to anadromous production in the lower part of the creek. Five of the adult steelhead that were sampled at the Big Bear Creek weir were assigned to the resident reference group, suggesting that they were the offspring or recent descendants of resident *O. mykiss* in the headwaters. It appears that some proportion of the Big Bear Creek headwater residents may undergo smoltification, although the mechanism driving that occurrence is unknown. High spring flows in Big Bear Creek may flush headwater residents downstream and below Big Bear Falls; those residents would then experience the same local environment as the anadromous population, perhaps leading to smoltification and an anadromous life history. Many studies have demonstrated that residency versus anadromy is a heritable trait in *O. mykiss* (Thrower et al. 2004; Doctor et al. 2014), but the ability of offspring from resident or anadromous parents to either smolt or residualize indicates that life history shows plasticity as well as sensitivity to the local environment (Zimmerman and Reeves 2000; Zimmerman et al. 2009; Christie et al. 2011; Courter et al. 2013).

The present study adds to the growing body of literature illustrating that the reproductive contribution of resident life history forms may be critical for the maintenance of genetic diversity in anadromous salmonid populations. This may be especially true in areas where anadromous fish abundance is low due to natural or anthropogenic influences (Courter et al. 2013). The life history diversity represented by resident individuals makes salmonid populations more resilient (Hilborn et al. 2003; Bisson et al. 2009; Greene et al. 2009; Schindler et al. 2010) and is thus believed to buffer against extinction (Hilborn et al. 2003). Snake River basin steelhead are listed as threatened under the ESA, whereas resident *O. mykiss* occurring in sympatry with steelhead do not receive ESA protection. This study and work by other researchers illustrate the importance of resident *O. mykiss* to the persistence and diversity of steelhead populations.

We advise caution in interpreting and applying 1 year of barrier passage results. We documented steelhead passage at Big Bear Falls during particular flow conditions, and we have documented considerable annual variation in streamflow within the Big Bear Creek drainage. Migration past the falls may only be achievable under certain flow regimes; varying flow conditions in subsequent years may reduce or enhance adult steelhead passage at the falls. In addition, there are still questions—outside the scope of this study—regarding the decrease in juvenile steelhead production above the falls. Additional years of data describing flow at Big Bear Falls and *O. mykiss* habitat use within areas surrounding and above the falls would further elucidate steelhead passage and habitat use.

We determined that Big Bear Falls is not a complete barrier to steelhead migration. However, based on the genetic analyses presented here and based on previous telemetry work

conducted in the Big Bear Creek drainage, we believe that the falls still plays a role in *O. mykiss* distribution and density and acts to limit gene flow in the drainage. We also expect that spatial differences and variation in environmental conditions experienced by resident and anadromous *O. mykiss* populations further promote their observed genetic isolation. We know that *O. mykiss* production within the 20 km above the falls is significantly lower than production below the falls. Three potential scenarios could explain the limited steelhead production above Big Bear Falls: (1) adult steelhead passage at the falls is limited, (2) survival of juvenile steelhead that rear above the falls is poor, or (3) juvenile steelhead that are spawned above the falls later emigrate below the falls to rear. Improvements in adult passage at Big Bear Falls would address the first of these potential scenarios by increasing steelhead access to habitat above the falls. However, without continued habitat restoration efforts that address additional limiting factors (e.g., reduced late-summer streamflow), the full potential of steelhead production in the Big Bear Creek drainage may not be realized.

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